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TB Lipidomics—The Final Frontier

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Lipids play critical roles in the biology of *Mycobacterium tuberculosis*, a notorious pathogen of man, but a systems-level approach to study these molecules have lagged behind other "omics" approaches. In this issue, Layre et al. describe the development of a powerful lipidomics technique and creation of a comprehensive database that address this need.

Species of the order Actinomycetales, which include Streptomyces and Mycobacteria, are unique in their amazing capacity for synthesizing a wide array of hydrophobic lipids and secondary metabolites. Perhaps the most notorious actinomycete, *Mycobacterium tuberculosis*, is a pathogen that has coevolved with its human host for millennia and still exerts an enormous toll on global health (World Health Organization, 2011). We have known from studies that date back to the 1950s that *M. tuberculosis* is rich in lipids (Sorkin et al., 1952), many of which are on the surface of the complex cell wall and exert profound effects on bacterial interactions with the environment as well as provide barrier functions to the cell (Brennan and Nikaido, 1995). Indeed, the elucidation of the *M. tuberculosis* genome in 1998 reflected the bacterium's substantial lipid biosynthetic capacity (Cole et al., 1998). Subsequent genetic and biochemical studies have since shown that some of the many lipid-associated genes can be placed into biosynthetic pathways, some of which are critical for virulence in animal models of infection. However, much remains unknown about the composition of the *M. tuberculosis* lipidome. In this issue, Layre et al. (2011) report an impressive technological achievement of lipidomics, and provide an unprecedented and comprehensive catalog of the lipidic metabolites of this important pathogen. This work, in combination with another group taking a similar approach (Sartain et al., 2011), represents an important milestone that will catalyze our understanding of the role of lipids in *M. tuberculosis* biology.

Layre et al. (2011) overcame a number of obstacles inherent in such an ambitious endeavor. Previous lipididomic studies with *M. tuberculosis* have used high resolution mass spectrometry (MS) or liquid chromatography mass spectrometry (LC-MS) but these methods were limited in the number of lipid species detected by several problems presented by the *M. tuberculosis* lipidome. First, the sheer number of species in the *M. tuberculosis* lipidome results in mass spectra peak overlap. This problem is partially solved by using high-resolution mass spectrometers (Jain et al., 2007), which can distinguish many lipids in complex crude extracts, but many ions still have overlapping spectra and thus cannot be distinguished. Another problem derives from the fact that in complex mixtures, some lipid species suppress the ionization of others, preventing their detection. Other studies utilizing LC-MS to separate lipids prior to MS were developed to examine only a few specific lipids and lacked the ability to separate other classes of mycobacterial lipids (Low et al., 2009), and global LC-MS methods developed for other bacteria poorly separate the abundant apolar lipids found in *M. tuberculosis*. Layre et al. (2011) overcame these limitations by developing a single-step method to effectively separate these species, resulting in a relatively straightforward protocol to globally monitor *M. tuberculosis* lipids. This has revealed over 100,000 MS peaks, representing 5,000 lipid species that can be reliably and reproducibly detected from bacteria grown in standard culture.

The second problem limiting lipidomic approaches in *M. tuberculosis* is the lack of a database cataloging the myriad of unique lipid species synthesized by mycobacteria. For eukaryotic cells, the LIPID MAPS consortium of lipidomics researchers has generated a powerful database that includes many resources, such as the structures and annotations of biologically important lipids as well as common protocols, that serve as useful tools for the research community (Schmelzer et al., 2007). Unfortunately, although this database contains information about common lipids such as glycerophospholipids and triacylglycerols, these databases obviously lack entries about the many unique lipids found in Mycobacteria, such as sulfolipids and phenolic glycolipids. Indeed, of the 58 lipid families already identified in *M. tuberculosis*, 40 have no counterparts in eukaryotic cells or gram-negative bacteria. Therefore, Layre et al. (2011) have also established a database similar to LIPID MAPS, termed MycoMass, which serves to match ions identified by MS to known chemical structures. This database will serve as a powerful tool for the study of *M. tuberculosis*, and as more structures are identified from *M. tuberculosis* and other lipid-rich bacteria, we envision that this tool will become of increasing benefit to the greater bacterial research community.

One of the benefits of this comprehensive approach is that it represents an important step in elucidating the chemical and structural entirety of the *M. tuberculosis* cell. The fact that only 20% of the ions detected by the authors matched known structures in the MycoMass database, however, indicates there are still many lipids produced by *M. tuberculosis* that remain unidentified. The comprehensive dataset of ions

detected will certainly aid in elucidating their structures, identifying their biosynthetic pathways, and determining their role in *M. tuberculosis* biology.

Importantly, the quantitative and reproducible data generated from this LC-MS approach will allow for future comparative studies to probe dynamic changes in the global lipidome. It is clear that changes in lipid content allow for *M. tuberculosis* to adapt to a variety of different conditions, including upon encountering stress (Cunningham and Spreadbury, 1998) and during infection (Kondo et al., 1970). The technology developed by Layre et al. (2011) will allow for a systems approach to study these types of dynamic changes. Indeed, the authors have demonstrated that this method can sensitively detect mycobacterial species even in extracts from *M. tuberculosis*-infected tissue "contaminated" with eukaryotic lipids. Understanding the changes that occur to the lipidome during infection or conditions that mimic infection such as reactive oxygen or nitrogen stress, low iron, and hypoxia, will be important to understand the role of lipids in virulence and the metabolic changes that occur during infection.

Approaches to globally monitor nucleic acids, proteins, and the water-soluble metabolites within cells have revolutionized our understanding of *M. tuberculosis* biology. The absence of similar methodologies to monitor hydrophobic lipids has been a glaring deficiency, especially given the important roles of these molecules during infection. The lipidomic platform pioneered by Layre et al. (2011) will allow researchers to finally probe this final frontier of the mycobacterial cell.

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A Wnt Inhibitor with a Twist

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Although the clinical safety of compounds targeting the core components of the Wnt signaling pathway remains to be determined, a simple in vivo chemical screen identifies small molecules that inhibit Wnt signaling in a cell type-specific manner (Ni et al., this issue of *Chemistry & Biology*).

The Wnt/β-catenin signaling pathway is undoubtedly one of the most prominent biological pathways. In 1987, Roel Nusse's group showed that the mouse mammary oncogene *int-1* was the homolog of the *Drosophila* segment polarity gene *wingless* (Rijsewijk et al., 1987). Thereafter, the name "Wnt," derived from the combined names of wingless and int-1, rightly signifies its myriad roles in regulating embryonic development and the homeostasis of adult tissues. In addition, aberrant Wnt signaling may cause cancers and other diseases (Clevers, 2006). Although inhibitors that target Wnt signaling ubiquitously may seemingly have the broadest utility, their potential for pleiotropic effects on adult tissues remains a concern. Conceivably, inhibitors that target cell type-specific components of the Wnt pathway may have better safety profiles. Why has such a compound not turned up in the screens that have been performed? Is discovery of tissue-specific Wnt inhibitors possible? In this issue, Ni et al. (2011) provide the first example of a tissue-specific Wnt inhibitor and demonstrate its potential for expanding cardiac progenitor cells.

Myocardial infarctions cause significant clinical problems in Western society. Thus, cardiogenic compounds are sought after for they may reactivate cardiac progenitors and repopulate infarcted myocardium. Several signaling pathways have been implicated in the genesis of cardiac progenitors from pluripotent stem cells. Moreover, a number of targeted and screening approaches for the identification of cardiogenic compounds have been reported (Hao et al., 2008;